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# Single- and multifrequency models for bioelectrical impedance analysis of body water compartments

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**Gudivaka, R., D. A. Schoeller, R. F. Kushner, and M. J. G. Bolt.** Single- and multifrequency models for bioelectrical impedance analysis of body water compartments. *J. Appl. Physiol.* 87(3): 1087–1096, 1999.—The 1994 National Institutes of Health Technology Conference on bioelectrical impedance analysis (BIA) did not support the use of BIA under conditions that alter the normal relationship between the extracellular (ECW) and intracellular water (ICW) compartments. To extend applications of BIA to these populations, we investigated the accuracy and precision of seven previously published BIA models for the measurement of change in body water compartmentalization among individuals infused with lactated Ringer solution or administered a diuretic agent. Results were compared with dilution by using deuterium oxide and bromide combined with short-term changes of body weight. BIA, with use of proximal, tetrapolar electrodes, was measured from 5 to 500 kHz, including 50 kHz. Single-frequency, 50-kHz models did not accurately predict change in total body water, but the 50-kHz parallel model did accurately measure changes in ICW. The only model that accurately predicted change in ECW, ICW, and total body water was the 0/∞-kHz parallel (Cole-Cole) multifrequency model. Use of the Hanai correction for mixing was less accurate. We conclude that the multifrequency Cole-Cole model is superior under conditions in which body water compartmentalization is altered from the normal state.

body composition; bioimpedance; deuterium; bromide; modeling

THE APPLICATION of bioelectrical impedance analysis (BIA) for the noninvasive assessment of human body composition was originally described by Hoffer et al. (13). Their work and most of the work that followed have used BIA for the prediction of total body water (TBW). Although the details of the predictive equations have varied, most have followed the paradigm of Hoffer et al. and utilized a serial resistance model to predict TBW (2).

Since that early work, the field of BIA of body composition has undergone a dramatic change. Central to this change has been the realization that the electrical properties of intracellular (ICW) and extracellular water (ECW) differ and that this difference might be exploited for the analysis of water compartmentalization in addition to TBW (9). Unfortunately, this change in the approach to BIA has also been accompanied by a proliferation of electrical models and resulting equations with which to estimate body water and compartments. Although it is unlikely that all the models can be correct because of the underlying differences in their assumptions, few comparative studies have been performed to identify the model or models that are the most accurate and precise for predicting body water compartments.

Although such an evaluation might appear simple, it is complicated by the fact that BIA does not provide a direct measurement of body composition. Instead, the BIA electrical signal must be correlated with a criterion measure of body composition to develop a predictive equation that relates the signal to the volume of water in the compartment of interest. Furthermore, this process is complicated by the fact that ECW, ICW, and TBW are highly intercorrelated (26). Therefore, excellent correlations are found between each of the water compartments and any number of representations of the bioelectrical data.

Two approaches have been used to try to reduce the confounding effect of the intercorrelation of body water compartments: *1*) performing the BIA validation studies in a group of subjects with abnormal compartmentalization of water secondary to disease (14) and *2*) performing the BIA validation studies in a group of subjects before and after an intervention that alters body water compartmentalization (7). Our present study uses the latter paradigm. The aim of the present study was to compare and contrast the numerous BIA models by validating them against a within-individual change in water compartmentalization that was induced by acute interventions. These interventions were expansion of ECW by infusion of lactated Ringer solution and reduction in body water by the administration of a potent diuretic agent. The bioelectrical models investigated included seven BIA models based on fixed-frequency 50-kHz or multifrequency impedance analysis.

## METHODS

### *Subjects*

Twenty-eight adults were enrolled in the study. Data from one subject were eliminated from the study, because the TBW by dilution differed by >1.2 liters at 2 and 3 h after the intervention. There were 14 men and 13 women, including 2 African-Americans, 4 Asians, and 21 Caucasians. The subjects were young to middle aged, healthy, and nonobese (Table 1). Exclusion criteria included a history of metabolic disease or any current treatment with prescription drugs. The protocol was approved by the University of Chicago Internal Review Committee, and all subjects gave informed, written consent.

The 27 subjects were divided between two protocols. One protocol involved 19 subjects who were studied on two occasions. Subjects fasted from 8 PM and refrained from vigorous exercise for 24 h before reporting to the Clinical Research Center (CRC) at 7 AM. Subjects wore a gown, and

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Table 1. *Subject characteristics*

| | Men ($n = 14$) | Women ($n = 13$) |
|---|---|---|
| Age, yr | 28 ± 8 | 26 ± 6 |
| Weight, kg | 83 ± 14 | 64 ± 10 |
| Height, cm | 181 ± 7 | 165 ± 8 |
| BMI, kg/m$^2$ | 25.2 ± 3.2 | 23.4 ± 3.0 |
| TBW, kg | 44.2 ± 6.3 | 30.6 ± 3.8 |
| ECW, kg | 15.7 ± 3.2 | 12.2 ± 1.8 |
| ICW, kg | 28.5 ± 3.7 | 18.4 ± 2.5 |
| $Ht^2/R_{50s}$, cm$^2$/Ω | 153 ± 25 | 99 ± 13 |
| $Ht^2/R_{50p}$, cm$^2$/Ω | 150 ± 25 | 97 ± 13 |
| $Ht^2/X_s$, cm$^2$/Ω | 1,058 ± 238 | 758 ± 145 |
| $Ht^2/X_p$, cm$^2$/Ω | 22.1 ± 4.1 | 12.7 ± 1.9 |
| $Ht^2/R_5$, cm$^2$/Ω | 128 ± 22 | 84 ± 12 |
| $Ht^2/R_{500}$, cm$^2$/Ω | 190 ± 30 | 120 ± 16 |
| $Ht^2/R_{ecf}$, cm$^2$/Ω | 119 ± 22 | 80 ± 12 |
| $Ht^2/R_{icf}$, cm$^2$/Ω | 80.5 ± 16.7 | 48.3 ± 7.6 |

Values are means ± SD. BMI, body mass index; TBW, total body water by deuterium; ECW, extracellular water by bromide; ICW, intracellular water by difference; $R_{50}$, series resistance at 50 kHz; $R_{50p}$, parallel resistance at 50 kHz; $X_s$, series reactance at 50 kHz; $X_p$, parallel reactance at 50 kHz; $R_5$, resistance at 5 kHz; $R_{500}$, resistance at 500 kHz; $R_{ecf}$, resistance extrapolated to 0 Hz; $R_{icf}$, resistance calculated for a parallel circuit and resistances extrapolated to 0 and infinite Hz ($R_\infty$).

weight was measured after the subjects voided. Height was measured with the subject barefoot with use of a wall-mounted stadiometer. Subjects drank a mixture of deuterium oxide and sodium bromide. Blood plasma specimens were obtained before and 3 h after the dose. An additional sample was collected at 2 h, and the tracer concentrations were compared at 2 and 3 h to identify subjects who failed to equilibrate (difference >3%). Subjects were asked to void at the time of the 3-h sample. The volume of urine was measured, and the subjects were reweighed.

After the 3-h sample the subjects were randomized into one of two treatments: *1*) intravenous infusion of lactated Ringer solution at a rate of 0.25 ml·kg$^{-1}$·min$^{-1}$ for 2 h or *2*) oral administration of 2 mg of butamide, a potent loop diuretic agent (21). Blood plasma was collected at 2 h after treatment (5 h after administration of tracer), all urine loss was measured, and aliquots were saved. Subjects were reweighed at 2 h after treatment. Plasma and urine specimens were stored at −10°C. Subjects were given nothing by mouth other than the tracer dose until the end of the treatment. It was hypothesized that these treatments would introduce an acute change in the physiological relationship between ECW and ICW.

Subjects returned to the CRC 1–3 wk later to receive the other treatment. The identical protocol was used, except the subject received the opposite treatment from that received in the first visit.

The remaining eight subjects were studied once under euvolemic conditions, as reported elsewhere (11). Subjects fasted from 8 PM and reported to the CRC at 7 AM. Height and weight were measured with the subject barefoot and wearing a hospital gown. TBW was measured by deuterium dilution, and ECW was measured by bromide dilution, with blood plasma collected before and 3 h after the dose. Subjects fasted during the 3-h equilibration period.

*TBW*

Body water was determined at 3 h after the dose (before treatment) and at the end of the 2-h treatment. Subjects received 0.14 g of deuterium oxide per kilogram of estimated TBW. The TBW was calculated from the deuterium dilution space ($N$) corrected for nonaqueous hydrogen exchange (23)

$$\text{TBW} = N/1.041$$

The deuterium dilution space was calculated at the time of final specimen collection from the change in deuterium abundance in body water, corrected for deuterium loss in urine and breath as well as tracer background in the infusate (postinfusion only) (23)

$$N = \frac{\text{d}}{\delta_f - \delta_b} - N_{out}\frac{\delta_{out} - \delta_b}{\delta_f - \delta_b} + N_{in}\frac{\delta_{in} - \delta_b}{\delta_f - \delta_b}$$

where d is the dose in moles, δ is the deuterium abundance, $N_{out}$ represents the moles of water lost as urine and breath vapor between the time of dosing and the final specimen, and $N_{in}$ represents the moles of water gained as infusate (postinfusion specimen only). The subscripts out, in, b, and f refer to the water output, water input, body water background, and final body water (postdose), respectively. Breath water lost was estimated as 34 and 14 ml/h, respectively (24). Pretreatment dilution space was calculated at 3 h after the tracer dose, and posttreatment dilution space was calculated at 5 h after the tracer dose.

The first term of the above dilution space equation is the typical dilution equation. The second and third terms correct for fluid intake and output (I/O) between the time of dose administration and blood sampling. These I/O values, while typically minor, were as large as 3 liters because of the treatments employed in this study. The inclusion of the tracer abundance values adjusted for small differences between fluids and amounted to corrections of 10–100 ml.

Deuterium was determined by isotope ratio mass spectrometry. Samples were untracentrifuged through a 50,000-Da exclusion filter (Amicon, Danvers, MA) to remove most protein. All other fluids were decolorized with dry carbon black (50 mg/10 ml) and passed through a 0.22-μm filter. Triplicate aliquots (2 μl) were distilled under vacuum into 6-mm-OD quartz tubes containing 40 mg of zinc reducing agent (Friends of Geology, Indiana University). The tube was flame sealed and heated to 500°C for 30 min. The resulting hydrogen gas was analyzed for deuterium abundance with use of differential isotope ratio mass spectrometry (25). The relative precision of the TBW determination was 1.5% (0.6 kg).

*ECW*

The bromide dilution space was determined at 3 h after the dose and again at the end of the 2-h treatment. Blood was collected in dry heparin, stored briefly on ice, then centrifuged to separate the plasma. A loading dose of 5 mg of sodium bromide per kilogram of body mass was administered by mouth. Blood plasma was obtained again at 3 and 5 h (after the additional 2-h treatment). ECW at 3 and 5 h was calculated from the bromide dilution space ($N_{Br}$)

$$\text{ECW} = 0.99 \times 0.95 \times 0.90 N_{Br}$$

where 0.99 is the fraction of water in ultrafiltered plasma, 0.95 is the Donnan equilibrium correction, and 0.90 is the estimated correction for penetration of bromide into the intracellular space (3, 16). The fraction of water in ultrafiltered plasma was gravimetrically determined in four freeze-dried plasma samples after passage through a 50,000-Da exclusion filter. The bromide dilution space was calculated

from the change in bromide concentration in plasma

$$N = \frac{\mathrm{d}}{[\mathrm{Br}]_f - [\mathrm{Br}]_b} - N_{out}\frac{[\mathrm{Br}]_{out} - [\mathrm{Br}]_b}{[\mathrm{Br}]_f - [\mathrm{Br}]_b} + N_{in}\frac{[\mathrm{Br}]_{in} - [\mathrm{Br}]_b}{[\mathrm{Br}]_f - [\mathrm{Br}]_b}$$

where the symbols are as defined above, except bromide concentration ([Br], mol/l) is used in place of the deuterium abundance. During the initial 3-h equilibration, the only adjustment was that for urinary loss. During the additional 2-h treatment, the bromide space was adjusted for continued urinary loss and, in the case of the lactated Ringer treatment, the difference between the bromide concentration in baseline plasma and the infusate.

Bromide concentration was measured by HPLC with use of the technique of Miller et al. (18). Briefly, plasma was filtered using a 50,000-Da Centricon exclusion filter. A 10-μl aliquot was introduced via a fixed-volume injection loop after a 30-μl flush. The bromide was isolated by liquid chromatography on a 250 × 4.6-mm Partisil SAX-10 ion-exchange column (Whatman, Clinton, NJ). A 30 mmol/l aqueous $KH_2PO_4$ mobile phase at 1 ml/min was used. Bromide was detected at 200 nm by use of a variable-wavelength ultraviolet detector calibrated against gravimetric sodium bromide standards prepared fresh each week. The relative precision of the ECW determination was 4% (0.7 kg).

### *BIA*

Electrode placement was proximal, as described by Scheltinga et al. (22). This was used in preference to typical wrist/ankle electrode placement, because the proximal placement is less sensitive to temperature and orthostatic effects (11). After the electrode site was cleaned with isopropyl alcohol, electrode patches (7.6 × 1.9 cm) with self-adhesive conducting gel (IS 4000, Xitron Technology, San Diego, CA) were attached to the dorsal surface of the right foot and right hand for current injection. Detector electrodes were attached to the dorsal surface of the right leg, with the proximal edge 1 cm distal to the center of the kneecap, and on the dorsal surface of the right forearm, with the proximal edge 1 cm distal to the midcrease of the antecubital fossa.

Subjects were instructed to walk around the room for 3 min before each BIA measurement to standardize the effects of orthostatic fluid shifts between the serial measurements (11). Electrodes were connected to the Xitron 4000B instrument (Xitron Technology) while the subjects were standing, and then the subjects were asked to lie down on a nonconductive, cloth-covered surface with their legs and arms abducted at ~45°. Bioimpedance spectra were obtained at 48 frequencies between 5 and 500 kHz in duplicate within 30 s after subjects assumed a supine position. The data were fit to the 0/∞-kHz parallel (Cole-Cole) model with use of the Xitron software package (version 1.00D). Values were averaged for the duplicate determinations.

### *Plasma Impedance*

The impedance of plasma at 50 kHz was measured in vitro with use of an acrylic cylinder with a 0.95-cm radius and aluminum plate electrodes at a height of 0.95 cm for the detector electrodes and 3 cm for the injector electrodes. A hand-held 0- to 100-Ω BIA instrument (model 101, RJL, Mt. Clemens, MI) was used for the in vitro resistance.

### *BIA Models*

*50-kHz serial model.* The 50-kHz serial model has been the most common model used for in vivo analysis of body water compartments by using the resistance ($R$) and reactance ($X$) as measured at 50 kHz. Its use is empirical and historic, because BIA instruments are configured such that the resistance and reactance readings correspond to a circuit in which a resistor and a capacitor are wired in series. On the basis of the original work by Hoffer et al. (13), the resistance index ($Ht^2/R_{50s}$, where Ht is height and s represents serial) is linearly correlated with TBW

$$\mathrm{TBW} = m\mathrm{Ht}^2/R_{50s} + c$$

where $m$ and $c$ are constants derived from linear regression of TBW on $Ht^2/R_{50s}$ in a reference population. The slope and intercept constants will be specific to each model. In this equation and those below, however, we used the generic symbols $m$ and $c$ for simplicity. Ht is utilized as a convenient estimate of circuit length. We use Ht to estimate circuit length for all the models.

Because reactance is related to the dielectric properties of cells, it might be assumed that ICW was linearly correlated with the reactance index ($Ht^2/X_{50s}$)

$$\mathrm{ICW} = m\mathrm{Ht}^2/X_{50s} + c$$

where $m$ and $c$ are model-specific constants derived from linear regression of ICW on $Ht^2/X_{50s}$ in a reference population.

*50-kHz parallel I model.* The 50-kHz parallel I model is a modification of the serial model that is based on a more physiological view of the human body. This model was proposed by Nyboer (19) and reported by others (1, 15). It is based on the assumption that the body behaves as if the resistance-capacitance circuit were arranged in parallel. The measured series resistance and reactance are therefore converted to their parallel equivalents

$$R_p = R_s + \frac{X_s^2}{R_s}$$

and

$$X_p = X_s + \frac{R_s^2}{X_s}$$

The parallel resistance ($R_{50p}$) and reactance ($X_{50p}$) are linearly related to TBW and ICW, respectively

$$\mathrm{TBW} = m\mathrm{Ht}^2/R_{50p} + c$$

and

$$\mathrm{ICW} = m\mathrm{Ht}^2/X_{50p} + c$$

where the model-specific constants are determined in a reference population.

*50-kHz parallel II model.* The 50-kHz parallel II model is very similar to the parallel I model described above, except it is based on the assumption that the parallel resistance pathway is exclusively extracellular rather than the total of ECW and ICW (14). Thus the assumptions are made that the parallel resistance and reactance are linearly related to ECW and ICW, respectively

$$\mathrm{ECW} = m\mathrm{Ht}^2/R_{50p} + c$$

and

$$\mathrm{ICW} = m\mathrm{Ht}^2/X_{50p} + c$$

where the constants are determined in a reference population.

*5/500-kHz serial model.* The 5/500-kHz serial model is an empirical model that was suggested by Deurenberg and Schouten (7) and other investigators (26). This dual-frequency model makes use of resistance measured at 5 and 500 kHz. These frequencies were selected because they were the lowest and highest frequencies that were available or reliable on common, commercial multifrequency impedance instruments. At 5 kHz the signal pathway is almost exclusively extracellular, because there is very little capacitive penetration of the signal into the intracellular volume (20), and thus it assumed that the resistance index is linearly related to ECW. At 500 kHz, there is extensive capacitive penetration of the intracellular compartment (20), and it is assumed that the resistance index at this frequency is linearly correlated with TBW. The equations are therefore

$$\text{TBW} = m\text{Ht}^2/R_{500s} + c$$

and

$$\text{ECW} = m\text{Ht}^2/R_{5s} + c$$

where the model-specific constants are determined in a reference population.

*5/500-kHz parallel model.* We have also investigated a variation on the above empirical model. The 5/500-kHz parallel variant recognizes that the specific resistivities (resistance per unit length of a conductor with a cross-sectional area of 1 $cm^2$) are quite different for extra- and intracellular fluids and that the resistance should be segregated into the extracellular ($R_{ec}$) and intracellular ($R_{ic}$) components by use of a parallel model (17)

$$\text{ECW} = m\text{Ht}^2/R_{ec} + c$$

and

$$\text{ICW} = m\text{Ht}^2/R_{ic} + c$$

where

$$R_{ec} = R_5$$

and

$$R_{ic} = (R_5 \cdot R_{500})/(R_5 - R_{500})$$

*0/∞-kHz parallel model.* The 0/∞-kHz parallel model is commonly called the Cole-Cole model. It is a more theoretical approach to deconvoluting the intra- and extracellular components of bioimpedance (5, 6). The approach is similar to the 5/500-kHz parallel model, in that the bioimpedance spectroscopy data are deconvoluted into an intra- and extracellular resistance, i.e., $R_{icf}$ and $R_{ecf}$, respectively. These resistances, however, are obtained from what is believed to be a more generalizable relationship for the β-dispersion portion of the bioimpedance spectrum (20). Specifically, the resistance and reactance data from a spectrum of frequencies between 5 and 500 kHz are fit to a semicircular plot by nonlinear, least-squares analysis, with allowance for depressed centroid (6). The values of resistance at 0 and ∞ frequency (the points where the curve intercepts the *x*-axis) are then calculated by extrapolation. $R_0$ is set to equal $R_{ecf}$, and $R_\infty$ is set to the parallel sum of $R_{ecf}$ and $R_{icf}$ ($1/R_\infty = 1/R_{ecf} + 1/R_{icf}$). $R_{ecf}$ and $R_{icf}$ indexes are then linearly related to ECW and ICW

$$\text{ECW} = m\text{Ht}^2/R_{ecf} + c$$

and

$$\text{ICW} = m\text{Ht}^2/R_{icf} + c$$

where the model-specific constants are determined in a reference population.

*0/∞-kHz parallel model with mixing.* The 0/∞-kHz parallel mixing model is frequently referred to as the Hanai model. Mixing theory predicts that the resistance of conducting solutions will increase as the concentration of nonconducting particles suspended in the solution increases (6). To a first approximation, this reflects the increase in the length of the conductive pathway, inasmuch as the current must curve around the nonconducting particles. Hanai developed a formula to describe this phenomenon in vitro. Application of the theory to in vivo uses, however, required modifications to the mathematical model (6). These included substitution of weight and density for mixture volume and height for conductor length.

The equation for the ECW is

$$\text{ECW} = k_{ecf}F_{ecf}$$

where

$$F_{ecf} = (\text{Wt}^{1/2}\text{Ht}^2/R_{ecf})^{2/3}$$

and Wt is weight, Ht is height, $R_{ecf}$ is zero-frequency resistance from the Cole-Cole model, and $k_{ecf}$ is a constant obtained by regressing the ECW measured by bromide dilution against $F_{ecf}$.

The equation for ICW is

$$\text{ICW} = r_{IE}\ \text{ECW}$$

where ECW is predicted as above and $r_{IE}$ (the ratio of ICW to ECW measured by dilution) is calculated by iteration with use of the following equation from Hanai

$$(1 + r_{IE})^{5/2} = r_{LH}[1 + (r_{IE}\, k_p)]$$

where $r_{LH}$ is the ratio of the resistance at zero to that at infinite frequency estimated from the Cole-Cole model and $k_p$ is a constant. This constant is determined by regressing $[(1 + r_{IE})^{5/2}/r_{LH} - 1]$ against $r_{IE}$.

### *Statistical Analysis*

Regression analysis was performed using two-way ANOVA (Minitab for Windows, Minitab, State College, PA) by using the BIA index ($\text{Ht}^2/\Omega$) and the appropriate water space for each of the models described in METHODS. Regressions were performed using the measurements of water spaces at the end of 5 h of treatment in the case of the 19 subjects in the diuretic-infusion protocol and at 3 h after tracer administration for the eight euvolemic subjects (Fig. 1). The effect of gender was identified using a discontinuous dummy variable (0 and 1) for ANOVA analysis. If a gender effect was observed, gender-specific prediction equations were developed.

The accuracy and precision for the predicted water spaces for each of the models were assessed using the change in water spaces across the two treatments in the 19 subjects, each studied with two treatments. The prediction equations were used to calculate the water spaces at the end of the 3-h equilibration. The BIA-predicted change in water volume during treatment was calculated from the difference between the predicted values at 3 and 5 h. These values were compared with the measured change in the water space with use of paired *t*-tests and Duncan's multiple-range adjustment. Differences in variance were compared with the *F*-test.

Values are means ± SD, except as noted. $P = 0.05$ was required for statistical significance.

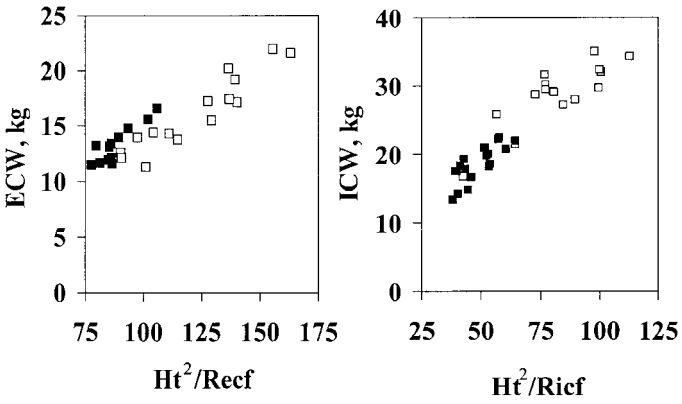


Fig. 1. Relationship between extracellular resistance ($R_{ecf}$) and intracellular resistance ($R_{icf}$) indexes calculated from 0/∞-kHz parallel (Cole-Cole) model and intracellular (ICW) and extracellular water (ECW) measured by dilution in men (□) and women (■). Ht, height.

## RESULTS

The subjects' characteristics in the euvolemic state are described in Table 1. The subjects were healthy, young to middle-aged adults with a body mass index between 19 and 32 kg/m$^2$.

Prediction equations for water spaces for all the bioelectrical models under consideration were developed in 19 subjects, each studied under two hydration states, and 8 subjects studied under euvolemic conditions. All the model-specific constants for the linear prediction equations developed for each of the BIA models were significant (Table 2). Gender was found to be significant for the prediction of water spaces for all models, except ECW with use of the 0/∞-kHz parallel (Cole-Cole) model and all three TBW models (Table 2). The correlations were generally higher for men, but this reflected the larger range of fluid spaces in men, inasmuch as the standard errors of the estimate (SEEs) were less in women than in men ($P < 0.05$). When adjusted for the larger average water spaces in men, the SEEs did not differ between genders, averaging ~8% for ECW and ICW and 5% for TBW. The SEEs for ECW among women did not differ between models. Among men the SEE was lowest for the 0/∞-kHz parallel with mixing (Hanai) model ($P = 0.05$). The SEE for the prediction of ICW among women tended to be lowest for the 0/∞-kHz parallel (Cole-Cole) and the 0/∞-kHz parallel with mixing (Hanai) models, but the difference was not significant. In men the SEE was lowest for the 0/∞-kHz parallel (Cole-Cole) model, and the SEE for the 50-kHz serial model was worse than for the other models ($P < 0.05$). The SEE for TBW tended to be lowest in the 5/500-kHz serial model, but the difference was not significant.

The treatments had the expected effect on hydration in the 19 subjects who received the intravenous infusion or diuretic (Table 3). The changes in TBW did not differ between the three criterion methods (body weight, intake/balance, and isotope dilution), averaging 1.7 kg for lactated Ringer infusion and −1.7 kg for the diuretic treatment. This was expected, because changes in body solids during 2 h are essentially limited to carbon lost as carbon dioxide, which we calculate to be ~0.1 g·kg$^{-1}$·h$^{-1}$, or ~0.020 kg for the average subject. Despite the comparability of the average changes, the precision of the change in TBW was consistently worse for the deuterium dilution measurement than either weight or water balance ($P < 0.01$). The increase in TBW by dilution after infusion ranged from 0.1 to 3.1 kg, and the decrease after diuretic treatment ranged from 0.9 to 2.6 kg. The standard deviation for the changes in TBW with treatment averaged 0.8 kg, which was not different from the expected precision, considering that the precision of the analysis was 0.6 kg [$0.6(2)^{1/2} = 0.8$ kg], suggesting that the range was inflated by random measurement error.

The change in ECW measured by dilution ranged from a loss of 0.5 kg to a gain of 3.7 kg after infusion and from a gain of 1.4 kg to a loss 2.7 kg after diuretic

Table 2. *Comparison of water space prediction equations for proximal bioelectrical impedance in the euvolemic state*

| Predictor Gender) | *c*, kg | *m*, kg·Ω·cm$^{-2}$ | $r^2$ | SEE, kg |
|---|---|---|---|---|
| | | *ECW* | | |
| $Ht^2/R_5$ | | | | |
| Men | −1.44 | 0.135 | 0.821 | 1.4 |
| Women | 1.17 | 0.131 | 0.738 | 1.0 |
| $Ht^2/R_{50p}$ | | | | |
| Men | −2.48 | 0.120 | 0.794 | 1.5 |
| Women | 0.71 | 0.118 | 0.700 | 1.0 |
| $Ht^2/R_{ecf}$ | | | | |
| Men | −0.06 | 0.133 | 0.806 | 1.4 |
| Women | 1.38 | 0.135 | 0.733 | 1.0 |
| All | 3.00 | 0.110 | 0.828 | 1.4 |
| Mixing† | | | | |
| Men | | | 0.905 | 1.0* |
| Women | | | 0.687 | 1.1 |
| | | *ICW* | | |
| $Ht^2/R_{ic}$ | | | | |
| Men | 10.5 | 0.295 | 0.723 | 2.1 |
| Women | 4.72 | 0.381 | 0.646 | 1.5 |
| $Ht^2/X_{50s}$ | | | | |
| Men | 19.8 | 0.008 | 0.205 | 3.5* |
| Women | 6.10 | 0.019 | 0.372 | 2.0 |
| $Ht^2/X_{50p}$ | | | | |
| Men | 11.16 | 0.77 | 0.911 | 2.2 |
| Women | 5.33 | 1.03 | 0.888 | 1.5 |
| $Ht^2/R_{icf}$ | | | | |
| Men | 12.8 | 0.191 | 0.573 | 2.6 |
| Women | 6.38 | 0.25 | 0.593 | 1.6 |
| Mixing† | | | | |
| Men | | | 0.590 | 2.5 |
| Women | | | 0.663 | 1.5 |
| | | *TBW* | | |
| $Ht^2/R_{50s}$ | 6.32 | 0.248 | 0.941 | 2.3 |
| $Ht^2/R_{50p}$ | 6.48 | 0.252 | 0.922 | 2.4 |
| $Ht^2/R_{500}$ | 6.57 | 0.199 | 0.948 | 2.0 |

Equations are of the following form: $mHt^2/R + c$, where $m$ is slope and $c$ is intercept. SEE, standard error of estimate; $r^2$, Pearson correlation constant; see Table 1 legend for definition of other abbreviations. *SEE differs from others within the same gender, $P < 0.05$. †Predictive equations for 0/∞-kHz parallel with mixing model are not of the form $y = mx + c$. For comparison, however, dilution volume was regressed against predicted volume, and $r^2$ and SEE are included.

Table 3. *Changes in specified variables introduced by each treatment*

| | Lactated Ringer Solution | Diuretic |
|---|---|---|
| Weight, kg | 1.59 ± 0.41 | −1.64 ± 0.31 |
| Intake/balance, kg | 1.62 ± 0.36 | −1.60 ± 0.18 |
| TBW, kg | 1.90 ± 0.96 | −1.82 ± 0.50 |
| ECW, kg | 1.59 ± 1.06 | −0.98 ± 0.98 |
| ICW, kg | 0.31 ± 1.38 | −0.83 ± 0.89 |
| $Ht^2/R_{50s}$, $cm^2/\Omega$ | 2.65 ± 0.92 | −2.00 ± 0.64 |
| $Ht^2/R_{50p}$, $cm^2/\Omega$ | 2.64 ± 1.11 | −2.08 ± 0.63 |
| $Ht^2/X_{50s}$, $cm^2/\Omega$ | 1.86 ± 0.68 | −1.49 ± 0.78 |
| $Ht^2/X_{50p}$, $cm^2/\Omega$ | 0.21 ± 0.50 | −0.08 ± 0.83 |
| $Ht^2/R_5$, $cm^2/\Omega$ | 1.54 ± 0.53 | −1.20 ± 0.29 |
| $Ht^2/R_{500}$, $cm^2/\Omega$ | 2.14 ± 1.56 | −1.69 ± 0.78 |
| $Ht^2/R_{ecf}$, $cm^2/\Omega$ | 1.63 ± 0.57 | −1.31 ± 0.28 |
| $Ht^2/R_{icf}$, $cm^2/\Omega$ | −0.03 ± 0.54 | −0.05 ± 0.85 |

Values are means ± SD. See Table 1 legend for definition of abbreviations.

treatment. We do not consider this large range to be physiological, because it exceeds that of the change in TBW. This range reflects the poorer precision of the ECW measurement (0.8 vs. 0.6 kg). Indeed, the standard deviation for the changes in ECW averaged 1.0 kg, which is not different from the expected standard deviation [$0.8(2)^{1/2} = 1.1$ kg], again suggesting that the range was inflated by random measurement error.

From tracer dilution, we calculated the ratio of ECW to TBW of fluid gain during the infusion to be 1.01 ± 0.63, indicating that the change was largely extracellular. The ratio of ECW to TBW in the fluid lost during diuretic treatment was 0.54 ± 0.56, indicating that ECW and ICW were altered. The precisions of the measured ratio of ECW to TBW in the fluid gained or lost, however, were poor, and the ratios ranged from −0.3 to 2.1 for infusion and from 0.9 and 1.4 for diuretic. Although the means of these ratios were physiologically reasonable, the range was unphysiological, i.e., individual subjects who appeared to lose ICW during infusion of lactated Ringer solution and subjects who gained ICW during treatment with a diuretic. The observation that the standard deviation for the changes in TBW and ECW is not larger than the measurement errors suggests that the large range results from the propagation of errors associated with measuring a small change in two large values.

Because the random error in the tracer dilution measurements was likely to limit the evaluation of the BIA models, the individual tracer dilution values were not used as the criterion method for the measurement of change in water spaces. Instead the criterion value for change in TBW during treatment was defined as $\Delta TBW = \Delta Wt$. The criterion method for change in ECW was defined as $\Delta ECW = f\Delta TBW = f\Delta Wt$, where f is the mean value of $\Delta ECW/\Delta TBW$ for the treatment. The criterion method for change in ICW was defined as $\Delta ICW = \Delta TBW - \Delta ECW = (1 - f)\Delta Wt$.

The conductivity of plasma was altered slightly by the treatments. In vitro resistances at 50 kHz averaged 116.2 ± 2.7 Ω after lactated Ringer infusion and 121.2 ± 4.3 Ω after diuretic treatment ($P < 0.05$). These changes in resistance expressed as a fraction of pretreatment resistance were −1.6 ± 2.3 and 1.6 ± 3.5%, respectively.

Table 4. *Effect of treatment on plasma composition*

| | Diuretic | | Lactated Ringer Solution | |
|---|---|---|---|---|
| | Baseline | Posttreatment | Baseline | Posttreatment |
| $Na^+$, mmol/l | 140.6 ± 1.8 | 139.6 ± 2.3 | 141.4 ± 1.8 | 140.7 ± 2.4 |
| $K^+$, mmol/l | 4.1 ± 0.6 | 3.9 ± 0.2 | 3.9 ± 0.2 | 4.1 ± 0.3 |
| Hct, % | 40.4 ± 3.7 | 43.5 ± 4.0* | 40.0 ± 4.6 | 36.8 ± 3.7* |
| Albumin, g/dl | 4.2 ± 0.3 | 4.7 ± 0.4* | 4.1 ± 0.3 | 3.8 ± 0.3 |

Values are means ± SD; $n = 12$. Hct, hematocrit. *Significantly different from baseline.

It was hypothesized that the change in plasma conductivity was due to a change in protein concentration secondary to the treatment. Plasma cation concentrations did not change with treatment, but plasma albumin concentration and hematocrit did change (Table 4). Because viscosity alters the conductivity of ionic solutions, we performed an in vitro experiment to determine whether the changes in conductivity for physiological changes in protein concentration were large enough to account for the difference in the conductivity of the plasma specimens that we observed. Albumin was mixed with lactated Ringer solution, and the resistance was measured in vitro as a function of albumin concentration (Fig. 2). Near physiological concentrations, a 1 g/dl change in albumin concentration resulted in a 1.3% change in resistance, which is similar to the change observed after the in vivo treatments. With the assumption that the changes in resistance observed in the plasma compartment were representative of the treatment effects on the electrical properties of body fluids, we adjusted values to remove this artifact. The individual predicted changes in ECW and TBW by BIA were increased or decreased by 1.6% of total ECW, respectively, for diuretic and infusion treatment. This adjustment was applied only for the extracellular pathway, because we had no means of accessing whether there was a similar artifact for the intracellular resistance.

The individual errors in predicted change in ECW, ICW, and TBW were calculated for each model. The predicted changes differed from the measured changes for at least one compartment for all but the 0/∞-kHz

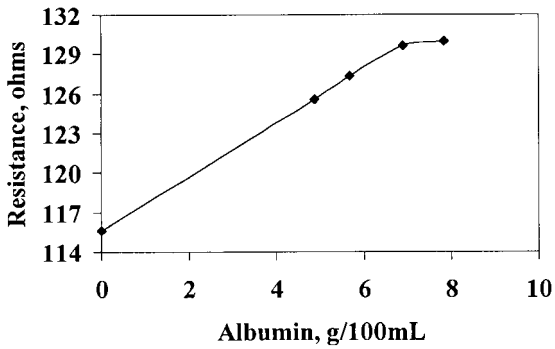


Fig. 2. In vitro resistance of an electrolyte solution (lactated Ringer solution) increases with increasing concentration of albumin.

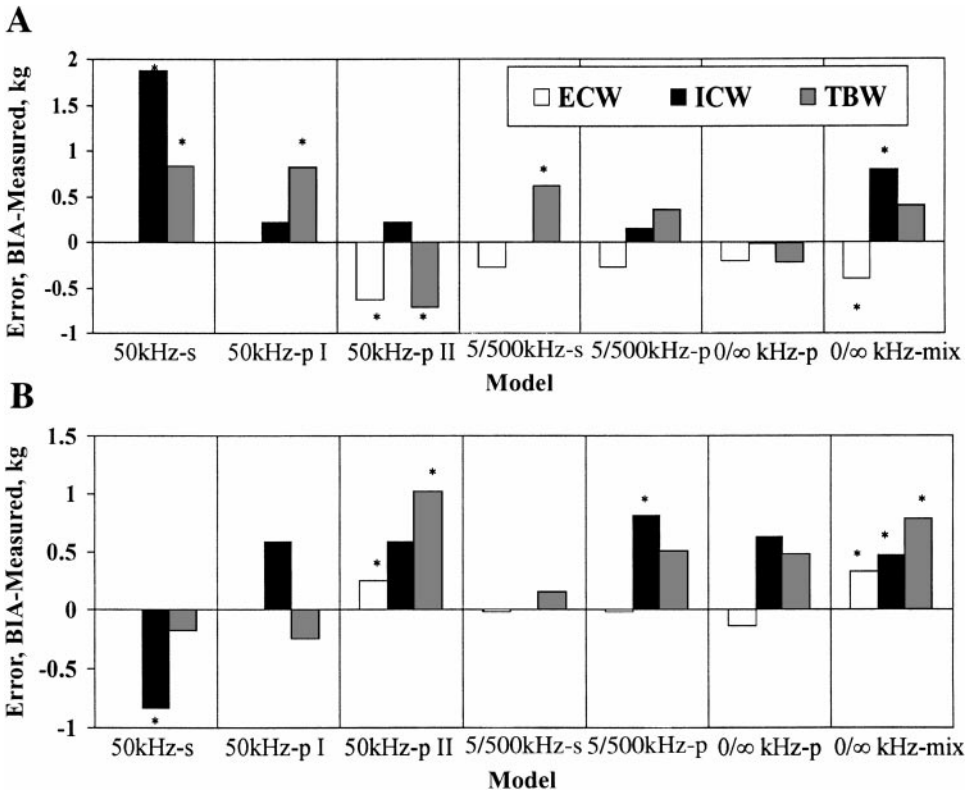


Fig. 3. Accuracy and precision for predicting change in water compartmentalization after treatment with diuretic (*A*) or lactated Ringer infusion (*B*) for each model. A positive error indicates that bioelectrical impedance analysis (BIA) overestimated change in water mass. TBW, total body water; 50-kHz-s, 50-kHz serial model; 50-kHz-p I and 50-kHz-p II, 50-kHz parallel I and II models; 5/500-kHz-s and 5/500-kHz-p, 5/500-kHz serial and parallel models; 0/∞-kHz-p and 0/∞-kHz-mix, 0/∞-kHz parallel and mixed models, respectively. * Significant error in predicted change in fluid weight, $P < 0.05$.

parallel (Cole-Cole) model (Fig. 3). The individual data for this model are shown in Fig. 4. The average accuracy and precisions for the predicted vs. measured changes for the 0/∞-kHz parallel (Cole-Cole) model were 0.2 ± 0.4 liter for ECW, 0.3 ± 0.7 liter for ICW, and 0.1 ± 0.7 liter for TBW. TBW was calculated from the sum of the ECW and ICW predictions. The 0/∞-kHz parallel with mixing (Hanai) model, which uses the Cole-Cole model as an intermediate, worsened the accuracy.

The single-frequency, 50-kHz serial and parallel models failed for TBW, although the parallel model approached good accuracy with a modest residual. The failure of the 50-kHz parallel II model was the prediction of ECW change. The 50-kHz parallel II model did predict change in ICW accurately (average residual = 0.4 ± 0.7 liter).

## DISCUSSION

On the basis of the assessment of within-subject changes in water compartmentalization, the 0/∞-kHz parallel (Cole-Cole) model is the most accurate model for the analysis of water compartmentalization in adults. The success of the 0/∞-kHz parallel (Cole-Cole) model can probably be attributed to the fact that the physiology of the body is best represented by the equivalent electrical circuits that correspond to the model; i.e., ECW and ICW are the major electrical conductors in the body, and they reside adjacent to each other in a parallel arrangement, with the ICW being isolated from the ECW by a nonconducting membrane similar to the insulating material within a capacitor. Conversely, the failures of the other models are not surprising when the equivalent electrical circuits of the models are compared with the physiology of the body.

The 50-kHz serial model assumes that the extra- and intracellular pathways are composed of a resistor and a capacitor in series and that the resistance and reactance are additive. From a physiological point of view, however, it appears that the intra- and extracellular pathways run side by side and thus constitute a parallel circuit. Thus the expectation is that the conductances ($1/R$) of the pathways will be additive and that the combined impedance of the two pathways will be less than either alone. This relationship, however, is not immediately apparent from the measurement of the electrical properties of the human body, because impedance analyzers measure resistance and reactance as though the circuit were a series circuit.

The 50-kHz parallel model improves on the series model in this regard, because the assumption is that the intra- and extracellular pathways are in parallel. The 50-kHz parallel I model, however, was built on the erroneous assumption that the resistance at 50 kHz was proportional to TBW. The 50-kHz frequency is too low for current to fully penetrate the body cell, a phenomenon that is demonstrated by the continuing decrease in resistance as frequency increases above 50 kHz (9). Thus the change in resistance with change in water space is overly sensitive to changes in ECW alone. This is evident in the results of our lactated Ringer infusion study, in which the change in body water occurred only in the ECW. The infusion increased ECW by an average of 1.3 liters or ~9%, whereas it only

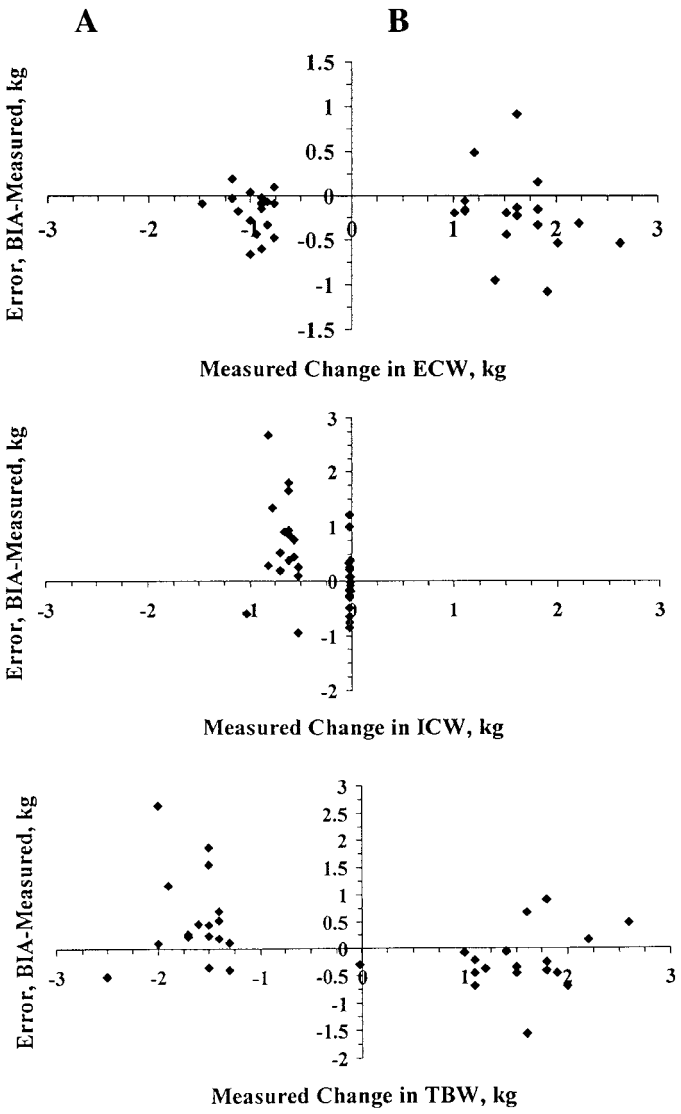


Fig. 4. Individual residuals between predicted change in body water compartments for 0/∞-kHz parallel (Cole-Cole) model and change in ECW, ICW, and TBW measured from weight change and treatment-specific ratio of ECW to TBW in subjects treated with diuretic (*A*) and lactated Ringer solution (*B*).

increased TBW by 3%, because the volume of ICW was unchanged. The parallel resistance decreased by 8%, which is similar to the 9% change in ECW, but not the 3% change in TBW, indicating that the resistance is a function of ECW and not TBW. This erroneous assumption was recently corrected by Kotler et al. (14), who applied the parallel model with the assumption that resistance was a function of ECW and reactance was a function of ICW. In our hands, however, the 50-kHz parallel II model failed for the assessment of ECW. This probably reflects the partial penetrance of the ICW at this frequency (20). Thus the model overpredicts change when the ECW is altered out of proportion to the ICW, as it was during the lactated Ringer infusion. The model, however, was accurate for ICW, and this is consistent with the excellent performance for estimating body cell mass (14).

The 5/500-kHz parallel model is a semiempirical model that improves on several of the above-described problems. It correctly assumes that the intra- and extracellular pathways are in parallel and that the low-frequency signal is a function of ECW. The model also correctly assumes that the signal travels through the ECW and ICW at high frequencies. The model fails, however, because it assumes that the specific resistances of the intra- and extracellular pathways are equal, when in fact the ICW has a much higher specific resistance than the ECW. This is primarily due to the high concentration of dissolved protein. Viscous fluids dramatically impede ion movement and thus increase the specific resistance.

The 0/∞-kHz parallel with mixing (Hanai) model similarly avoids the above erroneous assumptions and is similar to the 0/∞-kHz parallel (Cole-Cole) model, except it includes an adjustment for the presence of nonconducting material within the body. However, the model assumes that cells behave like nonconducting spheres suspended in a conducting medium, resulting in a lengthening of the current path as it winds around the cells. This is not the case, because much of the body cell mass is composed of skeletal muscle cells, which are not spherical, but elongated cells that are arranged in parallel along the axis of the limbs and thus in parallel to the current flow. Because of this, their concentration has a much smaller effect on the effective resistance of the ECW pathway (8). The Hanai model thus overcorrects for the mixing effect.

The present study also demonstrates the limitations of correlation analysis alone for testing the validity of BIA for the measurement of body water and its compartments when the subjects are euvolemic (Table 2). Most of the models demonstrated a high correlation between the various resistance index terms and their respective water space, as has been previously reported (7). Thus most of the models appeared valid when cross-sectional studies of healthy subjects were performed. Indeed, among healthy subjects, the difficulty has not been demonstrating that bioelectrical impedance correlates with water spaces but, rather, that it is specific for ECW, ICW, or TBW. This inability is due to the high degree of intercorrelation between ICW, ECW, and TBW in healthy individuals. Thus any impedance parameter that correlates with one of the water compartments will correlate almost equally well with the other water compartments, without necessarily being a specific measure of that compartment.

This high degree of intercorrelation between water compartments was the driving rationale for our use of interventions that alter the hydration status of the body and thus might alter the ratio of ICW to ECW. Although both treatments did alter this ratio, the infusion model with lactated Ringer solution was particularly effective in reducing the influence of the intercorrelation between water compartments.

Despite these advantages of this validation model, we found that we were limited by the precision of the isotope-dilution criterion methods for measuring the change in water compartments. The standard devia-

tions for the measurement of changes in ECW and TBW were 1.0 and 0.8 kg, respectively. We calculate that, with this precision in the criterion methods, we would have had to enroll four to eight times as many subjects in the validation to obtain the same statistical power obtained though the use of weight and group means of the ECW-to-TBW ratio as the criterion method. The rationale for the use of weight as the criterion method for change in TBW is quite sound for a period as short as that used in our study, because change in body solids is very minor in fasted individuals. We did, however, also have to partition this change into specific changes in ECW and ICW. We made the assumption that the ratio of change in ECW and ICW in each individual was the same as the group average. This assumption appears to be valid in our population, because the between-subject standard deviations for the change in fluid volumes were not greater than the estimated precision for measuring change in fluid volume on the basis of the precision of the dilution measurement. As such, the variation in the true or physiological distribution of changes in fluid after treatment must have been small relative to the measurement error, and, therefore, use of the individual values of ECW-to-TBW ratio would have overestimated the interindividual range of ECW and ICW changes.

The finding that most ECW and ICW models required gender-specific prediction equations was unexpected. None of the theoretical underpinning of the models predicts a gender effect. We found, however, that the inclusion of a dummy variable for gender proved significant and then generated separate prediction equations, which reduced the prediction error compared with a gender-nonspecific prediction equation. We can only speculate on the reason for the gender effect. One possible reason is that it is an artifact arising from the absence of significant overlap between the two groups of subjects. For example, because we employed a nonweighted regression even though the error decreased with smaller water mass, a smaller SEE will be observed in the women. This explanation, however, seems unlikely, because the intercepts do not appear to be randomly distributed around zero. Instead the intercepts always differ in the same direction for ECW and in the opposite direction for ICW. An even less likely regression methodology-related explanation would be that the relationship between water mass and BIA index is slightly nonlinear, resulting in different regression lines for different sections of the population. The data, however, do not support this finding with respect to the ECW models. The slopes for men and women are not different, as would be expected for linear fitting of a nonlinear relationship. Because the gender effect does not appear to be an artifact of the regression methodology, we speculate that it may be a physiological difference. Perhaps there is a systemic difference in the relative contribution of the limbs and trunk in men and women, and this leads to the gender difference in regression lines. It could also be due to gender differences in the conductivity of the body fluids, but this is not supported by the data. Gender differences in conductivity should result in different slopes for the genders, and this was not observed in the ECW models. A final possible explanation is that there is a gender difference in the bromide ICW penetration or mixing time that resulted in an artifact in the criterion methods. This last possible explanation is at least consistent with the observation that the gender differences between ECW and ICW cancel each other with respect to TBW, resulting in the absence of a gender effect. This gender effect is perplexing, and more research is required.

We compared the seven models that have been suggested for the analysis of body water compartmentalization using BIA at a single frequency or multiple frequencies. Among the single-frequency models, we find the 50-kHz parallel model for ICW to be valid. Among the multifrequency models, we find that the 0/∞-kHz parallel (Cole-Cole) model is the most precise and accurate model for the measurement of ECW and ICW. The accuracy of 0–200 ml and precision of 500–800 ml that we observed may exceed those that will be observed in an individual, because we were able to minimize the effects of other biological parameters that influence impedance independent of volume through the use of a short-term within-subject paradigm. These studies help extend the utility of BIA to measurements in subjects in which the ratio of ECW to ICW is altered, thus extending the utility of BIA beyond the limits proposed by the recent National Institutes of Health Technology Conference (2). On the basis of these results, we propose that the 0/∞-kHz parallel (Cole-Cole) model will be useful for the assessment of body water compartmentalization in diseased populations in which the normal ratio of ECW to TBW is altered. If, however, the goal is to assess small changes in fluid volumes, then it will be necessary to demonstrate that the results are not influenced by changes in specific conductivity or orthostatic effects (11). Although we found that 50-kHz series BIA was not valid under conditions of altered hydration, this does not negate the use of the model to predict absolute TBW in healthy subjects, because the errors are not likely to be significant as long as the ECW-to-ICW ratios are in the normal physiological range. The single-frequency series model only becomes invalid when the ECW-to-ICW ratio is altered, secondary to disease or drug treatment. When this occurs, the 0/∞-kHz parallel (Cole-Cole) model should be used.

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## REFERENCES

1. **Brown, B. H., T. Karatzas, R. Nakielny, and R. G. Clarke.** Determination of upper arm muscle mass and fat areas using

electrical impedance measurements. *Clin. Phys. Physiol. Meas.* 9: 47–55, 1988.
2. Bioelectrical impedance analysis in body composition measurement: National Institutes of Health Technology Assessment Conference Statement. *Am. J. Clin. Nutr.* 64: 524S–532S, 1996.
3. **Cheek, D. B.** Observations of total body chloride in children. *Pediatrics* 14: 5–10, 1954.
4. **Chumlea, W. C., S. S. Guo, D. B. Cockram, and R. M. Siervogel.** Mechanical and physiological modifiers and electrical impedance spectrum determinants of body composition. *Am. J. Clin. Nutr.* 64: 413S–422S, 1994.
5. **Cole, K. S.** *Membranes, Ions and Impulses: A Chapter of Classical Biophysics.* Berkeley, CA: University of California Press, 1972.
6. **De Lorenzo, A., A. Andreoli, J. Matthie, and P. Withers.** Predicting body cell mass with bioimpedance by using theoretical methods: a technology review. *J. Appl. Physiol.* 82: 1542–1558, 1997.
7. **Deurenberg, P., and F. J. M. Schouten.** Loss of total body water and extracellular water assessed by multifrequency impedance. *Eur. J. Clin. Nutr.* 46: 247–255, 1992.
8. **Epsteine, B. R., and K. R. Foster.** Anisotropy in the dielectric properties of skeletal muscle. *Med. Biol. Eng. Comput.* 21: 51–55, 1983.
9. **Foster, K. R., and H. C. Lukaski.** Whole-body impedance—what does it measure? *Am. J. Clin. Nutr.* 64: 388S–396S, 1996.
10. **Gretebeck, R. J., D. A. Schoeller, R. A. Socki, J. Davis-Street, E. K. Gibson, L. O. Schulz, and H. W. Lane.** Validation of the doubly labeled water method for subjects consuming isotopically enriched water. *J. Appl. Physiol.* 82: 563–570, 1997.
11. **Gudivaka, R., D. A. Schoeller, and R. F. Kushner.** Effect of body position, electrode placement and time on prediction of total body water by multifrequency bioelectrical impedance analysis. *Age Nutr.* 5: 111–117, 1994.
12. **Guyton, A. C.** *Textbook of Medical Physiology* (8th ed.). Philadelphia, PA: Saunders, 1991, p. 308–318.
13. **Hoffer, E. C., C. K. Meador, and D. C. Simpson.** Correlation of whole-body impedance with total body water volume. *J. Appl. Physiol.* 27: 531–534, 1969.
14. **Kotler, D. P., S. Burastero, J. Wang, and R. N. Pierson.** Prediction of body cell mass, fat-free mass, and total body water with bioelectrical impedance analysis: effects of race, sex and disease. *Am. J. Clin. Nutr.* 64: 489S–497S, 1996.
15. **Lukaski, H. C.** Biological indexes considered in the derivation of the bioelectrical impedance analysis. *Am. J. Clin. Nutr.* 64: 397S–404S, 1996.
16. **Manery, J. F.** Water and electrolyte metabolism. *Physiol. Rev.* 34: 334, 1954.
17. **Meijer, J. H., P. M. de Vries, H. G. Goovaerts, P. L. Oe, A. J. M. Donker, and H. Schneider.** Measurement of transcellular fluid shift during haemodialysis. 1. Method. *Med. Biol. Eng. Comput.* 27: 147–151, 1989.
18. **Miller, E. M., J. F. Cosgriff, and G. B. Forbes.** Bromide space determination using anion-exchange chromatography for measurement of bromide. *Am. J. Clin. Nutr.* 50: 168–171, 1989.
19. **Nyboer, J.** Workable volumes and flow concepts of biosegments by IPG. *Nutrition* 7: 396–409, 1972.
20. **Pethig, R.** Dielectric properties of body tissues. *Clin. Phys. Physiol. Meas.* 8, *Suppl.* A: 5–12, 1987.
21. *Physicians' Desk Reference.* Montvale, NJ: Medical Economics, 1998, p. 2441–2443.
22. **Scheltinga, M. R., D. O. Jacobs, T. D. Kimbrough, and D. W. Wilmore.** Alterations in body fluid content can be detected by bioelectrical impedance analysis. *J. Surg. Res.* 50: 461–468, 1991.
23. **Schoeller, D. A.** Hydrometry. In: *Human Body Composition*, edited by A. F. Roche, S. B. Heymsfield, and T. G. Lohman. Springfield, IL: Human Kinetics, 1996, p. 25–42.
24. **Schoeller, D. A.** Isotope dilution methods. In: *Obesity*, edited by P. Bjorntorp and B. N. Brodoff. Philadelphia, PA: Lippincott, 1992, p. 80–88.
25. **Schoeller, D. A., E. van Santen, D. W. Peterson, W. Dietz, J. Jaspen, and P. D. Klein.** Total body water measurement with $^{18}0$ and $^{2}H$ labeled water. *Am. J. Clin. Nutr.* 33: 2686–2693, 1980.
26. **Segal, K. R., S. Burastero, A. Chun, P. Coronel, R. N. Pierson, and J. Wang.** Estimation of extracellular water and total body water by multifrequency bioelectrical impedance measurement. *Am. J. Clin. Nutr.* 54: 26–29, 1991.